

to light from the first point. In this manner, spatial information in the bead is transformed by the collection lens into angular information in the collection path. The spectral dispersing element acts on the collimated light such that different spectral components leave the spectral dispersing element at different angles, in a plane substantially orthogonal to the direction of the relative movement between the bead and the imaging system. In this manner, both spatial and spectral information in the bead are transformed into angular information. The imaging lens acts on the light from the dispersing element to transform different light angles into different positions on the detector. Spatial information is preserved by the system since light from the different positions in the bead is projected along a path 42 to different positions on the detector, in both axes. In addition, light of different spectral composition that originates from the bead is projected to different positions on the detector in an axis substantially orthogonal to the relative movement between the bead and the imaging system. In this manner, the spatial information from the bead is preserved, and spectral information covering a large bandwidth at high resolution is simultaneously collected.

When used for bead identification in accord with the present invention, this apparatus provides substantial utility in resolving reporter locations and spectra on the detector, even when the reporters are disposed in spatially close relationship within a bead. When spectral imaging occurs in the present invention, the spatial distribution of light in the bead is convolved with the spectral distribution of that light to produce the image of the bead at the detector. This convolution can result in blurring in the dispersion axis, depending on the spectral bandwidth of the light. Narrow spectral bandwidths will result in little or no blurring, depending on the spectral resolution of the system. In the present invention, it is contemplated that the spectral resolution will be approximately 3 nm per pixel, with a spatial resolution in object space of approximately 1 micron. However, the spatial and spectral resolution can be adjusted to match the requirements of a particular application and the values noted herein should not be considered as limiting on the scope of the present invention.

Alternate embodiments of the imaging system illustrated in FIGURE 2A and useful for imaging beads are shown in FIGURES 2C-4. These alternate embodiments differ in the number and orientation of various optical components, as described below, but generally function in the same manner as the imaging system of FIGURE 2A.

FIGURE 2C illustrates an imaging system 45 that includes a slit 52 used to prevent extraneous light from reaching pixelated detector 44. In imaging system 45, light 46 from bead 24 (or other object) is focussed by an objective lens 48 onto slit 52. Slit 52, as shown in FIGURE 2C, is sufficiently narrow to block light that is not focussed onto the slit by objective lens 48, thereby preventing extraneous light from

passing through the slit. Light 30', which has passed through the slit, is collected by collection lens 32, as discussed above, producing collected light 34. Collected light 34 is spectrally dispersed by prism 36 and is imaged by imaging lens 40 onto pixelated detector 44, also as discussed above. By excluding light other than that from object 24 from reaching pixelated detector 44, the detector produces an output signal that corresponds only to the actual images of an encoded bead, and the signal is not affected by the extraneous light that has been excluded. If not excluded in this manner, ambient light reaching pixelated detector 44 might otherwise produce "noise" in the output signal from the pixelated detector.

It should be noted that in the illustration of each of the imaging systems in FIGURES 2A and 2C, a light source has not been shown. These first two embodiments have been illustrated in their most general form to make clear that a separate light source is not required to produce an image of an encoded bead if the object is luminescent, i.e., if the object produces light. However, in most uses of the imaging apparatus for imaging beads, one or more light source(s) will be used to provide light that is incident on the bead being imaged. The location of the light sources substantially affects the interaction of the incident light with the bead (or other object) and the kind of information that can be obtained from the images on the pixelated detector.

In FIGURE 3, several different locations of light sources usable to provide light incident on bead 24 are illustrated. It should be understood, however, that light sources can be located at many other positions besides those shown in FIGURE 3. The location of each light source that is employed will be dependent upon the kind of imaging of the bead, and the kind of data for the bead, to be derived from the signal produced by the pixelated detector. For example, employing a light source 60a or a light source 60b, as shown in the figure, will provide light 58 that is incident on bead 24 and which is scattered from the bead into the optical axis of collection lens 32. The optical axis of collection lens 32 is at about a 90° angle relative to the directions of the light incident upon bead 24 from either light source 60a or 60b. In contrast, a light source 62 is disposed so that light 58 emitted from that source travels toward the bead in a direction that is generally aligned with the optical axis of collection lens 32, so that the image formed on detector 44 will not include light absorbed by bead 24. Light absorption characteristics of the bead can thus be determined by illuminating the bead using light source 62.

A light source 64 is disposed to illuminate bead 24 with light directed toward the bead along a path that is approximately 30-45° off the optical axis of collection lens 32. This light 58, when incident on bead 24, will be reflected (scattered) from

bead 24, and the reflected or scattered light will be imaged on detector 44. A more directly reflected light is provided by an epi light source 66, disposed so as to direct its light 58 toward a partially reflective surface 68 that is disposed so that a portion of the light is reflected through collection lens 32 and onto bead 24. The light reaching the
5 bead will be reflected from it back along the axis of collection lens 32 and will at least in part pass through partially reflective surface 68 to form an image of the bead on pixelated detector 44. Alternatively, a dichroic mirror may be employed instead of, and in the position of, partially reflective surface 68 to direct light from epi light source 66 to excite fluorescence or other stimulated emission from bead 24. Light emitted from
10 bead 24 at a different wavelength than the light source is then at least partially collected by collection lens 32 and passes through the dichroic mirror for spectral dispersion and detection by the detector.

Each of the light sources illustrated in FIGURE 3 produce light 58, which can either be coherent, noncoherent, broadband, or narrowband light, depending upon the
15 application of the imaging system for imaging beads. Thus, a tungsten filament light source can be used for applications in which a narrowband light source is not required. For applications such as stimulating the emission of fluorescence from reporters, narrowband light is preferred, since it also enables a spectrally-decomposed, nondistorted image of the bead to be produced from light scattered by the bead. This
20 scattered light image will be separately resolved from the reporters produced on pixelated detector 44, so long as the emission spectra of any reporters are at different wavelengths than the wavelength of the light. The light source can be either of the continuous wave (CW) or pulsed type. If a pulsed type illumination source is employed, the extended integration period associated with pixelated detection can allow
25 the integration of signal from multiple pulses. Furthermore, it is not necessary for the light to be pulsed in synchronization with the pixelated detector.

FIGURE 4 illustrates an arrangement that enables the imaging of a bead from two different directions, in order to distinguish features that would otherwise overlap when viewed from a single direction. A stereoscopic imaging system 70 in FIGURE 4
30 includes two pixelated detectors 44a and 44b, and their associated optical components, as discussed above in connection with the imaging system of FIGURE 2A.

By positioning the optical axes of collection lenses 32 for the two pixelated detectors so that they are spaced apart, for example, by 90°, it is possible to separately resolve the signature of reporter tags imaged from two or more reporters on at least one
35 of pixelated detectors 44a or 44b. If two or more reporters overlap in regard to the image produced on one of the detectors, they will be separately resolved in spectrally dispersed image produced on the other pixelated detector. Further, the use of two